Molecular Biology

LONG-TERM ARCHIVAL STORAGE OF *SALMONELLA TYPHIMURIUM* LT2 CULTURES LEADS TO GENOMIC AND PHENOTYPIC CHANGES

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Intensive studies on mutation rates in *Salmonella typhimurium* have taken place in our laboratory. These studies were made possible due to our unique collection of over 20,000 archival cultures. These auxotrophic mutant cultures have been sealed in agar stab vials at room temperature for over 40 years. This resource has provided us with the opportunity to examine the genetic and evolutionary changes. The imminent publication of the complete annotated genome of *Salmonella enterica* Serovar Typhimurium LT2 makes this a propitious time to examine the association of functional losses with chromosomal map positions. The long-term objective is to compare the characteristics of archival strains with the sequenced *S. typhimurium* and identify the fate of certain known genes. The specific aims are: [1] *To catalog phenotypic alterations among S. typhimurium survivors after decades in a sealed environment.* [2] *To correlate the phenotypic observations with genomic differences between archival survivors and the sequenced ancestral strain.* [3] *To identify and analyze cryptic and lytic phages isolated from archival collection, particularly those phages that may be involved in pathogenicity processes.* The experimental strategies for addressing these questions include conducting a census among survivors from different vials in order to score characteristics of the members of these populations using a battery of phenotypic screens. Screens that have taken place include: Scoring for metabolic changes using BioLog Phenotype Microarray™ (PM) plates followed by plating on MacConkey agar base supplemented with a variety of sugars, pulsed-field gel electrophoresis (PFGE), phage susceptibility, motility tests for chemotactic and flagellar gene losses, growth curves, and antibiotic sensitivity. Additional genomic and protein assays will identify additional mutations as well as large deletions, inversions and transpositions using restriction fragment analysis and PFGE. The phage recovered from the stored vials will be characterized to determine their identity or similarity with known phage, as well as their transduction capacity. Map positions of all chromosomal changes will be determined to identify any "use-it-or-lose-it" strategy for survival in a restricted environment. Not only the resulting data, but also cultures from the collection of over 20,000 isolates will continue to be available to all scientists with an interest in bacterial evolution and diversity.